Pharmacological characterization of the bifunctional opioid ligand H-Dmt-Tic-Gly-NH-Bzl (UFP-505)†

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Editor’s key points
- µ-Opioid (MOP) receptor activating opioids are good analgesics but have side-effects.
- Blocking the δ-opioid (DOP) receptor along with activating MOP reduces tolerance.
- This study characterized a novel opioid which activated MOP and blocked DOP receptors.
- The novel opioid UFP-505 was shown to be a MOP/DOP bifunctional opioid.

Background. While producing good-quality analgesia, µ-opioid (MOP) receptor activation produces a number of side-effects including tolerance. Simultaneous blockade of δ-opioid (DOP) receptors has been shown to reduce tolerance to morphine. Here, we characterize a prototype bifunctional opioid H-Dmt-Tic-Gly-NH-Bzl (UFP-505).

Methods. We measured receptor binding affinity in Chinese hamster ovary (CHO) cells expressing recombinant human MOP, DOP, κ-opioid (KOP), nociceptin/orphanin (NOP) receptors. For activation, we measured the binding of GTPγS to membranes from CHO-MOP, CHO-DOP, rat cerebrocortex, and rat spinal cord. In addition, we assessed ‘end organ’ responses in the guinea pig ileum and mouse vas deferens.

Results. UFP-505 bound to CHO-MOP and CHO-DOP with (binding affinity) pKi values of 7.79 and 9.82, respectively. There was a weak interaction at KOP and NOP (pKi 6.29 and 5.86). At CHO-MOP, UFP-505 stimulated GTPγS binding with potency (pEC50) of 6.29 and in CHO-DOP reversed the effects of a DOP agonist with affinity (pKB) of 9.81 (in agreement with pKi at DOP). UFP-505 also stimulated GTPγS binding in rat cerebrocortex and spinal cord with pEC50 values of 6.11–6.53. In the guinea pig ileum (MOP-rich preparation), UFP-505 inhibited contractility with pEC50 of 7.50 and in the vas deferens (DOP-rich preparation) reversed the effects of a DOP agonist with an affinity (pA2) of 9.15.

Conclusions. We have shown in a range of preparations and assays that UFP-505 behaves as a potent MOP agonist and DOP antagonist; a MOP/DOP bifunctional opioid. Further studies in dual expression systems and whole animals with this prototype are warranted.

Keywords: bifunctional opioids; morphine; opioid ligands; UFP-505

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Morphine is a gold standard analgesic acting at the µ-opioid receptor (MOP). Although a potent analgesic, long-term use of morphine is associated with a wide range of side-effects, including respiratory depression, constipation, tolerance, and dependence. While the potent antinociceptive/analgesic effects of morphine are attributed solely to its activity at the MOP receptor, questions were raised about the contribution of the δ (DOP) or κ (KOP) opioid receptors to morphine tolerance and dependence.

There is clear evidence for functional interaction between MOP and DOP such that DOP antagonism (receptor or genetic) reduces tolerance to MOP agonists.2–5 Interestingly, an elegant study by Hepburn and colleagues6 showed that although the administration of naltrindole (DOP antagonist) to chronically morphine-treated rats resulted in the attenuation of the morphine-induced antinociceptive tolerance and also in reduced withdrawal effects, it did not prevent the manifestation of tolerance of the morphine-induced respiratory depression. This selective ‘effect’ of naltrindole administration in morphine-treated rats (i.e. allowing the development of a ‘protective’ respiratory depression tolerance to morphine while at the same time reducing the morphine-induced antinociceptive tolerance) highlights the possibility that the mechanisms behind the antinociceptive tolerance of morphine may implicate MOP–DOP receptor interaction, whereas the mechanisms behind the respiratory depression tolerance do not (or at least not in the same way).
Moreover, Nitsche and colleagues\(^7\) have reported that although knock-out mice of the endogenous DOP receptor ligand preproenkephalin did not develop analgesic tolerance to morphine, they still manifested physical dependence, implying that morphine tolerance is genetically distinct or manifested distinctly from mechanisms of dependence. Additionally, in the same study, a similar tolerance-deficit profile to morphine has been shown in \(N\)-methyl-D-aspartate receptor-deficient mice, underlying the possibility that common mechanisms of the development of morphine tolerance also exist between opioid and non-opioid receptors. These interesting interactions have also been confirmed in a different study by Sharif and colleagues\(^8\) where intrathecal administration of metabotropic glutamate receptor-1 antisense oligonucleotides in rats resulted in an attenuation of morphine tolerance. Finally, based on studies that have shown a reduction in the development of tolerance to morphine by NOP receptor blockade or in NOP receptor knockouts, the co-administration of a MOP agonist and NOP antagonist has been proposed.\(^9\)

Collectively, these studies indicate that the pharmacology of opioid receptors is far more complicated than originally thought, possibly through linked mechanisms where the binding of a ligand to one opioid receptor (i.e. DOP) can affect the behaviour of another (i.e. MOP). This cross-talk between two different opioid receptors has been hypothesized to be partially responsible for the pharmacological subtypes observed\(^10\) and has been the focus of studies for a number of years, either by investigating the type of interaction among opioid receptors\(^11\)–\(^13\) or by investigating the pharmacology of ligands that bind to these receptors.\(^14\)–\(^15\) Strategies aimed at simultaneous targeting of MOP and DOP with a single molecule represent a possible alternative to morphine in terms of increased analgesia with reduced tolerance. We have recently reviewed the non-selective or bifunctional ligand strategy.\(^16\)

As part of an ongoing programme to design and explore single MOP agonist/DOP antagonist drugs,\(^17\)–\(^22\) we describe the pharmacological characteristics of our prototype compound H-Dmt-Tic-Gly-NH-Bzl, named here UFP-505\(^17\)–\(^23\) using Chinese hamster ovary (CHO) cells expressing recombinant human MOP/DOP/KOP/NOP (CHO\(_{hMOP}/CHO_{hDOP}/CHO_{hKOP}/CHO_{hNOP}\)), rat spinal cord and cerebral cortex, guinea pig ileum, and mouse vas deferens in a range of \(in\) \(vitro\) assays. An outline of our strategy is depicted in Table 1.

### Methods

Additional methodology can be found in the Supplementary material.

### Cell culture and tissue isolation

CHO cells were grown in an appropriate medium [F12(Ham)1X for the hMOP, hDOP, and hKOP cells and DMEM/F12(Ham)1:1 for the hNOP cells] containing 10% fetal bovine serum, 100 IU ml\(^{-1}\) penicillin, 100 \(\mu\)g ml\(^{-1}\) streptomycin, and 2.5 \(\mu\)g ml\(^{-1}\) fungizone, as described previously.\(^24\) All media contained \(L\)-glutamine. Stock cultures were additionally supplemented with 200 \(\mu\)g ml\(^{-1}\) G418 (a selection agent used with hMOP, hDOP, and hKOP cells) and with additional 200 \(\mu\)g ml\(^{-1}\) hygromycin B (an additional selection agent) for the hNOP cell. Cell cultures were kept at 37°C in 5% CO\(_2\)/humidified air and subcultured as required using trypsin/EDTA. Cells were used for experimentation as they approached confluence.

Healthy male albino rats (200–250 g) were killed by decapitation and their spinal cord tissue and cerebral cortex

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### Table 1

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Pharmacological characterization of the bifunctional opioid ligand UFP-505

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were isolated. Tissues were then stored at −80 °C and used later for membrane preparation as described below.

The guinea pig ileum and mouse vas deferens tissues were taken from male albino guinea pigs (300–350 g) and male Swiss mice (30–35 g), respectively. On the day of the experiment, the animals were killed by decapitation. From the mouse, the prostatic portion of the vas deferens was isolated and prepared according to Hughes and colleagues and from the guinea pig, a segment of the ileum (1.5–2 cm in length) was taken as described by Maggi and colleagues. In our study, tissues from a total of 10 rats, 12 guinea pigs, and 12 mice were used and are in accordance with the guidelines published in the European Communities Council directives (86/609/EEC).

Saturation-binding assay to determine receptor density

Membrane protein (70–100 μg) prepared as described in the Supplementary material was incubated in 0.5 ml volume of 50 mM Tris, 0.5% BSA, 10 μM of a variety of peptidase inhibitors (aminastatin, bestatin, captopril, phosphoramidon), and various concentrations of radioligand 3H-DPN for hMOP/ DOP/KOP or 3H-UFP-101 for hNOP for 1 h at room temperature. Non-specific binding (NSB) was defined in the presence of 10 μM naloxone for hMOP/DOP/KOP or 1 μM unlabelled UFP-101 for hNOP. Reactions were terminated and bound/free radioactivity were separated by vacuum filtration through polyethylenimine (0.5%)-soaked Whatman GF/B filters, using a Brandel harvester. Bound radioactivity was determined after 8 h extraction in ScintisafeGel (Wallac, Loughborough, UK) using liquid scintillation spectroscopy.

Displacement binding assay to determine ligand selectivity and binding affinity

Membrane protein (70–100 μg) was incubated as in saturation assays, but containing ~1 nM 3H-DPN for hMOP/DOP/ KOP or ~0.8 nM 3H-UFP-101 for hNOP and varying concentrations (10 μM−1 pM) of a range of displacer ligands. NSB was defined in the presence of 10 μM naloxone for hMOP/DOP/ KOP or 1 μM of N/OFQ for the hNOP preparations. Assay incubation time, reaction termination, and bound radioactivity were as in the saturation assays.

GTPγS assay to determine ligand functional activity

Membrane protein (70–100 μg) was incubated in 0.5 ml volume of 50 mM Tris, 0.2 mM EGTA, 1 mM MgCl2−6H2O 100 mM NaCl, 0.1% BSA, 0.15 mM bacitracin; pH 7.4 (with NaOH), for 1 h at 30 °C with gentle shaking, to which 10 μM peptidase inhibitors (aminastatin, bestatin, captopril, phosphoramidon), GDP (33 μM), and ~150 pM GTPγS were added. NSB was determined in the presence of non-radioisobellled 10 μM GTPγS. Reactions were terminated by vacuum filtration through dry Whatman GF/B filters, using a Brandel harvester. Bound radioactivity was determined as in the saturation assays. Endomorphin-1, DPDPE, and UFP-505 were included at various concentrations and combinations as described in the Results section.

Electrically stimulated isolated tissues to determine ligand functional activity

Bioassay experiments were performed as previously described for the mouse vas deferens (mVD) and guinea pig ileum (gpi). Tissues were suspended in 5 ml organ baths containing heated Krebs solution oxygenated with 95% O2 and 5% CO2, at 33 °C for the mouse vas deferens and 37 °C for the guinea pig ileum. Tissues were stimulated through two platinum ring electrodes with supramaximal rectangular pulses of 1 ms duration and 0.05 Hz frequency. A resting tension of 0.3 and 1 g were applied to the mouse vas deferens and guinea pig ileum, respectively. The electrically evoked contractions (twitches) were measured isotonically by means of Basile strain gauge transducers (Basile 7006, UgoBasile s.r.l., Varese, Italy) and recorded with a PC-based acquisition system (Power Lab, 4/25, ADInstruments, Australia). After an equilibration period of 60 min, the contractions induced by electrical field stimulation were stable. For the guinea pig ileum, dermorphin and UFP-505 were included at various concentrations and combinations, with or without the presence of the non-selective opioid receptor antagonist naloxone or the MOP-selective antagonist α-Phe-Cys-Tyr-ω-Trp-Orn-Thr-Pen-Thr-NH2 (CTOP), as described in the Results section. For the mouse vas deferens, DPDPE was used at various concentrations and combinations, with or without the presence of different concentrations of UFP-505, as described in the Results section.

Data analysis

All data are presented graphically as mean (SEM) from (n) experiments with derived tabulated data as mean (so). Concentration–response curves were analysed by non-linear regression using GraphPad Prism V5.0 software (San Diego, CA, USA). In saturation-binding assays, the receptor density (Bmax) and radioligand equilibrium dissociation constant (pKD) were obtained from saturation-binding isotherms and semi-log transformations of specific binding data. In displacement binding assays, the 50% displacement of specific binding was corrected for the competing mass of radiolabel and the pKD values were obtained by using the Cheng and Prusoff equation (IC50/(1+[L]/KD)), where IC50 is the effective concentration of the ligand that displaces 50% of the radioligand, [L] the concentration of the radioligand used, and KD the dissociation constant of the radioligand that was determined in this study.

For the GTPγS experiments, data are presented as percentage stimulation of GTPγS binding from basal activity (un-stimulated). The pKD value of UFP-505 was calculated using the formula pKD = −log10(CR-1)/[UFP-505]), where CR is the ratio of the EC50 of DPDPE in the presence and absence of UFP-505.

For the electrically stimulated guinea pig ileum assays, cumulative concentration–response curves of dermorphin
and UFP-505 were performed in the absence or presence of antagonists. For the electrically stimulated mouse vas deferens assays, cumulative concentration–response curves of DPDPDE were produced in the absence or presence of 1, 10, and 100 nM UFP-505. A Schild linear regression plot was used to calculate the antagonist affinity of UFP-505.

Results

Saturation-binding assays

The binding of ³H-DPN and ³H-UFP-101 to all CHO membranes was concentration-dependent and saturable (Fig. 1 and Supplementary material). The $B_{max}$ values for ³H-DPN or ³H-UFP-101 at CHO$_{MOg}$, CHO$_{DOP}$, CHO$_{KOP}$, and CHO$_{NOP}$ can be seen in Table 2. The $p_Kd$ value of ³H-DPN was lower in hDOP, as reported previously.12

Displacement binding assays

The binding of ³H-DPN and ³H-UFP-101 was displaced in a concentration-dependent manner by the respective reference ligands (EM1, naltindrole, nBNI, and N/OFQ) (Fig. 2A–D). Estimated $p_Ki$ values are shown in Table 2. UFP-505 displaced ³H-DPN binding to MOP and DOP receptors with $p_Ki$ values of 7.79 and 9.82 (n=5), respectively. The $p_Ki$ of UFP-505 for the MOP receptor was similar to that of the endogenous ligand EM1 (8.09). Fentanyl and morphine binding curves were included in CHO$_{MOg}$ for comparison ($p_Ki$; fentanyl 7.35, morphine 8.55). The rank order $p_Ki$ of the four MOP agonists was morphine > EM1 > UFP-505 > fentanyl. For the DOP receptor, the UFP-505 affinity was the same as that of the highly selective antagonist naltindrole (9.82). The affinity for KOP and NOP receptors was negligible (<6.3). The rank order $p_Ki$ of UFP-505 for the opioid receptors was DOP $\gg$ MOP $\gg$ KOP $>$ NOP.

Functional assays

Both UFP-505 and endomorphin-1 stimulated the binding of GTP$_y$S to membranes prepared from CHO$_{MOg}$ cells, with similar efficacy values (stimulation of UFP-505 and EM1 was 128% and 129.3%, respectively) and potency values ($p_{EC_{50}}$

![Graph](https://via.placeholder.com/150)

**Figure 1** Saturation-binding assays performed on CHO$_{MOg}$, CHO$_{DOP}$, CHO$_{KOP}$, and CHO$_{NOP}$ cell membranes, with increasing concentrations of tritiated diprenorphine (³H-DPN) or tritiated UFP-101 (³H-UFP-101). Log-transformed specific binding isotherms are shown and were used to determine the maximum receptor binding capacity ($B_{max}$) and equilibrium dissociation constant ($p_Kd$) in the respective membranes (see also Table 2 and Supplementary material Fig. S1). Single representative curves are presented here (from n=5). Radioligand binding affinity ($p_Ki$) and receptor density ($B_{max}$; fmol/mg protein) values are summarized in Table 2.
of UFP-505 and EM1 was 6.37 and 6.38, respectively) (Fig. 3A). At the DOP receptor (Fig. 3B), UFP-505 was essentially inactive as an agonist (6.2% stimulation at the highest concentration), where the reference ligand and full DOP agonist, DPDPE, stimulated GTPγS binding by 31.3% with a potency of 8.34 (n = 3). In our hands, DOP responses are always lower than MOP. At the
DOP receptor, 10 nM UFP-505 produced a parallel rightward shift in the DPDPE concentration–response curve (Fig. 3C) yielding a $pK_b$ value of 9.81, which is in agreement with the $pK_i$ value determined in the $^3$H-DPN binding assays (Table 2).

In the rat spinal cord (mixed opioid expression) functional assay (Fig. 4A), the pEC$_{50}$ of endomorphin-1 and UFP-505 were found to be 6.69 and 6.53, respectively. The efficacy values were 25.6% and 19.5%, respectively. In the rat cerebral cortex (mixed opioid expression) functional assay (Fig. 4B), the pEC$_{50}$ of endomorphin-1 and UFP-505 were found to be 6.59 and 6.11, respectively, while the $E_{\text{max}}$ values were 49.2% and 30.2%, respectively.

**Stimulated isolated tissues**

In the electrically stimulated guinea pig ileum (MOP receptor-rich), presented as percentage of control twitch in the absence and presence of 10 nM naloxone and 100 nM of the MOP-selective antagonist CTOP. (a) The dermorphin curve was shifted to the right by naloxone and CTOP. (a) Similarly, the UFP-505 curve was shifted to the right by naloxone and CTOP. All data are mean (SEM) from at least five experiments and are also summarized in Table 2.

DOP receptor, 10 nM UFP-505 produced a parallel rightward shift in the DPDPE concentration–response curve (Fig. 3C) yielding a $pK_b$ value of 9.81, which is in agreement with the $pK_i$ value determined in the $^3$H-DPN binding assays (Table 2). In the rat spinal cord (mixed opioid expression) functional assay (Fig. 4A), the pEC$_{50}$ of endomorphin-1 and UFP-505 were found to be 6.69 and 6.53, respectively. The efficacy values were 25.6% and 19.5%, respectively. In the rat cerebral cortex (mixed opioid expression) functional assay (Fig. 4B), the pEC$_{50}$ of endomorphin-1 and UFP-505 were found to be 6.59 and 6.11, respectively, while the $E_{\text{max}}$ values were 49.2% and 30.2%, respectively.

**Stimulated isolated tissues**

In the electrically stimulated guinea pig ileum (Fig. 5A), the selective MOP receptor agonist dermorphin inhibited the electrically induced twitches with a pEC$_{50}$ of 9.47. This effect was antagonized by naloxone (pK$_b$ 8.80) and CTOP (pK$_b$ 8.29).

In the electrically stimulated mouse vas deferens (Fig. 6A), DPDPE inhibited the electrically induced twitches with a pEC$_{50}$ value of 8.07. Addition of various concentrations of UFP-505 shifted the DPDPE curve to the right, with a Schild analysis producing an antagonist potency value (pA$_2$) of 9.15. The analysis showed a slope value of 0.96 ([0.04], $r^2=0.992$). Similarly for the same tissue (Fig. 6B), naloxone shifted the control DPDPE curve (pEC$_{50}$ 8.23) to the right with a pEC$_{50}$ of 7.78 and a pK$_b$ of 8.26.

**Discussion**

We have shown that UFP-505 has affinity for the MOP receptor comparable with the endogenous ligand endomorphin-1 and to agonists used in the clinic; morphine and fentanyl.
UFP-505 displayed higher affinity at DOP and was very weak at KOP and NOP. In functional screens (upstream in GTP\(^{35}\)S), UFP-505 was an MOP agonist (in CHO\(_{hMOP}\) and rat neuronal tissue) and DOP antagonist (in CHO\(_{hDOP}\)). In these latter assays, UFP-505 also produced a small increase in GTP\(^{35}\)S binding that seemed to vary with batches. This is in agreement with previously published values in the limited functional study of Balboni and colleagues. With regard to DOP antagonism, it is reassuring that the antagonist \(pK_a\) calculated at CHO\(_{hDOP}\) agrees with the \(pK_i\) estimated in radioligand displacement assays. In downstream functional assays, UFP-505 also behaved as an MOP agonist (gpI) and DOP antagonist (mVD) and again the antagonist \(pA_2\) was close to \(pK_i\) determined for the (human) DOP.

Multiple receptor selectivity (subjectively defined as the property of a novel ligand that is designed to bind selectively to more than one receptor; thus having a different meaning from non-selectivity) has been the focus of an increasing number of studies in recent years. Rational drug design and structure–activity relationship studies have been used to evaluate the pharmacology of various ligands that act simultaneously either on two different types of opioid receptors, on an opioid receptor and a non-receptor protein (i.e. tramadol), or on an opioid receptor and an unrelated receptor. Collectively, MOP and DOP receptor trafficking properties, especially receptor desensitization and endocytosis upon ligand binding, has been shown to play a key role in the manifestation of opioid tolerance and dependence and therefore renders multiple targeting as an important tool for drug design strategies.

UFP-505 (H-Dmt-Tic-Gly-NH-Bzl, see structure in Table 1) is a bifunctional pseudotripeptide and is best described as a prototype to enable a directed structure activity analysis to be effected.

Balboni and colleagues demonstrated that the C-terminal insertion of a further aromatic moiety on the Dmt-Tic pharmacophore increases the MOP affinity. Moreover, the distance between the Dmt-Tic pharmacophore and a third aromatic nucleus is an important chemical modification able to convert the Dmt-Tic from a highly potent DOP antagonist into a potent DOP agonist or into ligands with mixed DOP and MOP opioid properties.

In previous preliminary data for UFP-505, an increased affinity for the DOP receptor (\(pK_i\: 10.50\)) and the MOP receptor (\(pK_i\: 9.80\)) in the rat brain assay was reported. In the same study, in the functional bioactivity assay, UFP-505 had a high antagonist potency (\(pA_2\: 9.25\)) in the mouse vas deferens and a \(pEC_{50}\) of 8.57 in the guinea pig ileum.

Additionally, Balboni and colleagues have recently published further pharmacodynamic data on UFP-505 using CHO cells expressing human opioid receptors, as part of a study that explored the properties of various Dmt-Tic-containing ligands. UFP-505 has shown the most promising profile of the ligands studied, with an MOP affinity (\(pK_i\: 8.66\)) and DOP affinity (\(pK_i\: 9.58\)). These values were calculated based on radioligand \(pK_d\) values of \(^3\)H-DAMGO, \(^3\)H-naltrexone, and \(^3\)H-U69593 (9.25, 9.47, and 10, respectively). In the same study, UFP-505 displayed significant MOP potency (\(pEC_{50}\: 7.72\)) and stimulation (\(E_{max}\)) of 84%, a higher DOP antagonist potency (\(pEC_{50}\: 8.89\)) and a very weak DOP stimulation of 6.8%.

As a link between the simple and genetically pure hMOP/ DOP preparations used to determine a basic binding profile, we assessed the functional activity (GTP\(^{35}\)S) in a relevant mixed opioid population; the rat spinal cord and cerebrocortical membranes. We demonstrated efficacy, which will be the MOP component with higher values in the cortex than cord. This is most likely explained by expression differences and agrees with previously published work. Potency estimates at CHO\(_{hMOP}\) agree with these data. However, a drawback of this preparation is that the presence of MOP precludes simple use in a GTP\(^{35}\)S assay to estimate DOP antagonism.

Fig 6 Effects of DPDPE in the electrically stimulated mouse vas deferens (DOP receptor-rich), in the absence and presence of increasing concentrations of UFP-505 and 10 nM naloxone. (A) The DPDPE curve is shifted in parallel to the right by increasing concentrations of UFP-505 (1, 10, 100 nM). Data presented as percentage of control twitch. (a) Schild analysis of (A) produces a regression line with a slope of 0.96 (0.04) which represents competitive antagonism, whereas the extrapolation of the line when \(y=0\) represents the antagonist potency \(pA_2\) (logarithmic) 9.15. (C) In a similar preparation, naloxone shifted the DPDPE curve to the right. Data presented as percentage of control twitch. All data were produced from at least five experiments for each preparation and are summarized in Table 2.
As a final assessment of functional activity, we elected to use the well-characterized MOP-rich guinea pig ileum and DOP-rich mouse vas deferens tissues. These tissues are used as screening tools for MOP and DOP activity and represent a more intact preparation. The pEC50 for UFP-505 in gpl appears more potent than in CH0 and rat tissues. This may result from the use of disrupted and well-washed membranes and GTPγS binding assays compared with the use of intact guinea pig tissue. This supposition might be correct based on the reasonably close agreement in the antagonist potency for this compound at DOP (i.e. antagonist interaction should be unaffected by the presence/absence of any intracellular mediators). However, simple species differences might also be important.

Conclusion
From the above, we conclude that the bifunctional ligand UFP-505 shows full MOP agonist activity, comparable with fentanyl, and is a potent DOP antagonist. To the best of our knowledge, UFP-505 is one of the few most efficacious and potent bifunctional MOP agonists/DOP antagonists16 17 23 which might prove useful in terms of reduced side-effect profile when compared with morphine. Further in vivo studies are now planned.

Supplementary material
Supplementary material is available at British Journal of Anaesthesia online.

Declaration of interest
R.G. and G.C. are members of a university spin-out company, University of Ferrara Peptides (UFPeptides) that is involved in the development of opioid ligands. N.D., J.M., S.M., D.J.R., and D.G.L. are collaborators with UFPeptides. D.G.L. holds a consultancy with Grunenthal GmbH and is a Director of the British Journal of Anaesthesia.

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